Method for Estimating the Magnitude of Chronic Effects of Cyanide on Listed Species

The first preference for quantitatively assessing chronic effects of cyanide on listed species would be to use data from chronic toxicity tests/studies with the species in question. If, however, chronic toxicity data are not available or are not suitable it may be necessary to estimate effects on listed species using surrogate species. For cyanide, species-specific chronic toxicity data are not available for the listed species for which "likely to adversely effect" (LAA) determinations have been made. To estimate the type and magnitude of effects for evaluation in the Biological Opinion (BO), some estimate of the chronic effects of cyanide on LAA listed species is needed. The approach taken here was adapted from the screening level assessment employed in the BE methodology. According to the BE methodology, if chronic data for the listed species are not available the chronic threshold for the listed species, i.e. the "Assessment Effects Concentration" or EC_A is estimated using data from surrogate species. The EC_A is intended to estimate the highest chemical concentration in water (aquatic species) or food (aquatic-dependent species) that would cause no adverse effect or would adversely affect an acceptably-small percentage of individuals within a specified species population. For chronic toxicity, the EC_A is based on the acute toxicity to the listed species, and the Acute to Chronic Ratio (ACR) of surrogate species. The ACR, for the purposes of the national BE methodology and here, is calculated as follows:

$$ACR = \frac{SS\ LC_{50}}{SS\ NOEC} \qquad (1)$$

Where: SS LC_{50} is the LC_{50} for the surrogate species

SS NOEC is the No Observable Effects Concentration for the surrogate species

EC_A's are estimated using the following equation:

$$EC_A = \frac{LS\ LC_{50}}{ACR} \qquad (2)$$

Where: LS LC_{50} is the LC_{50} for the listed species

The ACR and EC_A are graphically illustrated in Figures 1 and 2. The effects determination for the BE is based on a comparison of the EC_A for a listed species and the Criterion Continuous Concentration (CCC) for cyanide, 5.2 ug CN/L. If the EC_A is less than 5.2 ug CN/L the species is likely to be adversely affected and if the EC_A is greater than 5.2 ug CN/L the species is not likely to be adversely affected. The type of effect and its severity (beyond likely versus not likely) were not part of the BE, but that information is needed for the BO to help characterize the magnitude of effects to individuals, populations and the species as a whole. As mentioned above, the BE method was modified in order to estimate the magnitude of effect that would occur if the listed species was exposed to cyanide at the CCC.

Equations 1 and 2 can be combined by substituting equation 1 for the ACR term in equation 2:

$$EC_A = \frac{LS\ LC_{50}}{\frac{SS\ LC_{50}}{SS\ NOEC}}$$

Rearrange:

$$EC_A = \frac{LS\ LC_{50}}{SS\ LC_{50}} * SS\ NOEC \qquad (3)$$

Figure 3 illustrates how equation 3 can also be used to calculate the $EC_{A.}$ In this case the relative difference in sensitivity between listed species and surrogate species to acute exposures is used as a sensitivity adjustment factor to calculate the Chronic EC_A for the listed species from the surrogate species NOEC.

The level of effect occurring at the EC_A corresponds to the level of effect occurring at the surrogate species NOEC, and is intended to be acceptably low. However, the level of effect at the NOEC can vary between studies (refer to NOEC/LOEC discussion in the Cyanide BO) and thus the level of effect at the EC_A would vary as well. To more accurately reflect the level of effect associated with the EC_A, equation 3 can be rewritten in a more general form:

$$LS\ EC_X = \frac{LS\ LC_{50}}{SS\ LC_{50}} * SS\ EC_X \qquad (4)$$

Where:

LS EC_X is the Effects Concentration for the listed species that elicits a response of magnitude X, and;

SS EC_X is the Effects Concentration for the surrogate species that elicits a response of magnitude X.

For the Biological Opinion, we are interested in estimating how listed species are affected by cyanide when exposed at the CCC (5.2 ug CN/L). We can use equation 4 to do this by first setting the LS EC_X equal to the 5.2 ug CN/L then calculating the Effects Concentration for the surrogate species, SS EC_X, and finally estimating X (magnitude of effect) from the exposure – response relationship for the surrogate species:

First, set

$$LS\ EC_X = 5.2 \text{ ug CN/L}$$

Substitute in equation 4,

$$5.2 \text{ ug CN/L} = \frac{LS\ LC_{50}}{SS\ LC_{50}} * SS\ EC_X$$

Next, rearrange:

$$SS\ EC_X = \frac{SS\ LC_{50}}{LS\ LC_{50}} * 5.2 \text{ ug CN/L} \qquad (5)$$

Because the SS LC_{50} and LS LC_{50} are known (or estimated), setting the LS EC_X equal to the 5.2 ug CN/L allows for the calculation of SS EC_X. The SS EC_X is the effects concentration for the surrogate species that is equivalent to the effects concentration for the listed species at the 5.2 ug CN/L, after adjusting for differences in sensitivity between the surrogate and listed species based on the ratio of acute toxicities, i.e. SS LC_{50}/LS LC_{50}.

For example, the LC_{50} for fathead minnow, a surrogate species, is 138 ug CN/L and the estimated LC_{50} for the Maryland darter, a listed species, is 40 ug CN/L. Based on these values fathead minnows would be 3.45 times less sensitive than Maryland darters and the SS EC_X would be 17.9 ug CN/L, that is, 3.45 times higher than the CCC (5.2 ug CN/L). In other words, fathead minnows would have to be exposed to a concentration 3.45 times higher than the CCC to experience an effect, equal in magnitude, to the effect on the Maryland darter exposed at the CCC.

Once the SS EC_X is calculated, the magnitude of effect (X) can be estimated using the chronic exposure-response curve for the surrogate species (Figure 4). In this illustration the chronic toxicity data for the fathead minnow was fitted to a log-linear regression model. The magnitude of effect, X, at the SS EC_x (17.9 ug CN/L) can be estimated using this model. For fathead minnow, an SS EC_x of 17.9 ug CN/L corresponds to an effect of about 54%. Therefore, the CCC (5.2 ug CN/L) would correspond to an EC_{54} for the Maryland darter, or a 54% effect concentration.

This estimation method relies on two underlying assumptions: (1) that relative differences in sensitivity between surrogate species and listed species to acute exposures are good approximations of the relative differences in sensitivity to chronic exposures and (2) that the slope of the exposure-response curves for surrogate and listed species are reasonably similar.

Figure 1. Illustration of how the Acute to Chronic Ratio (ACR) is determined.

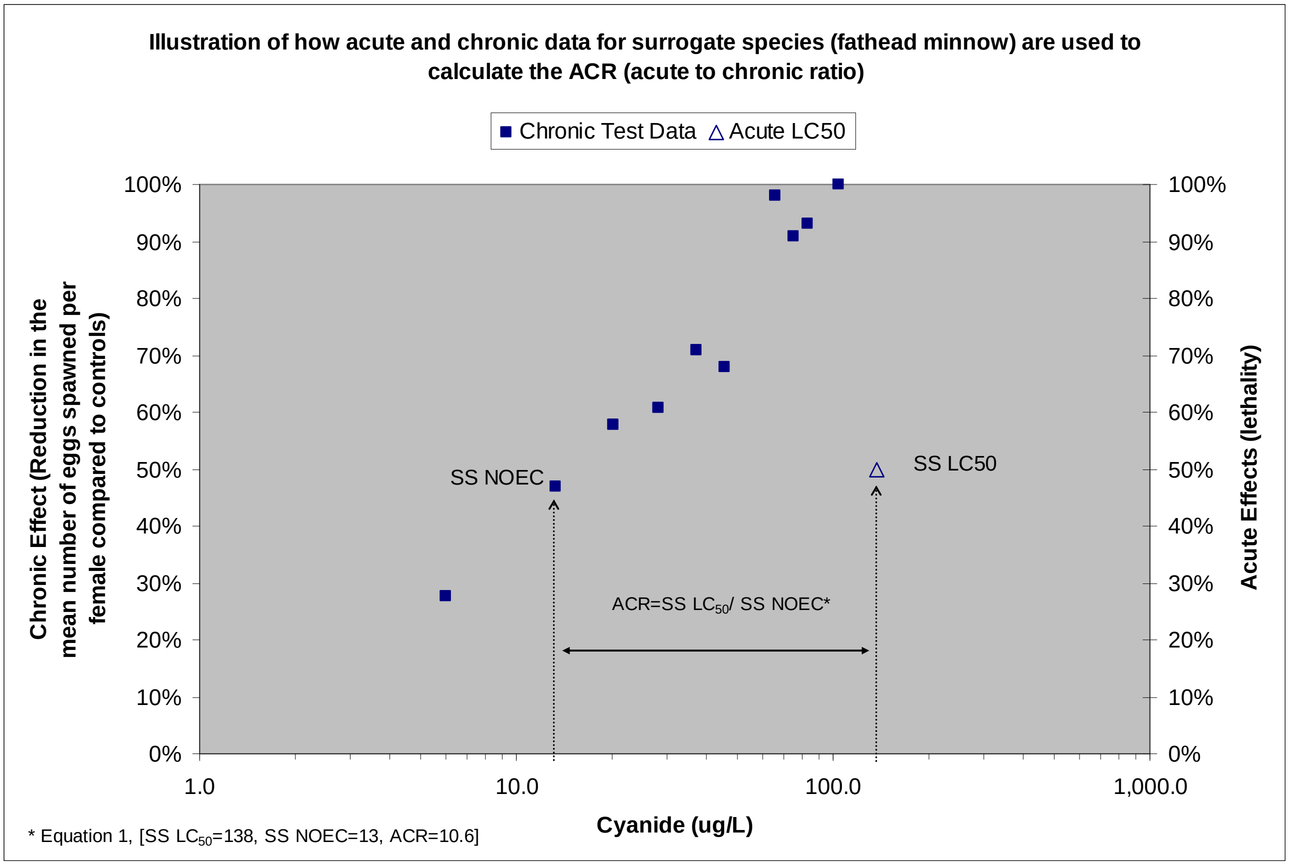


Figure 2. Illustration of how the ACR (Acute to Chronic Ratio) is used to estimate Chronic Assessment Effects concentrations (EC_A).

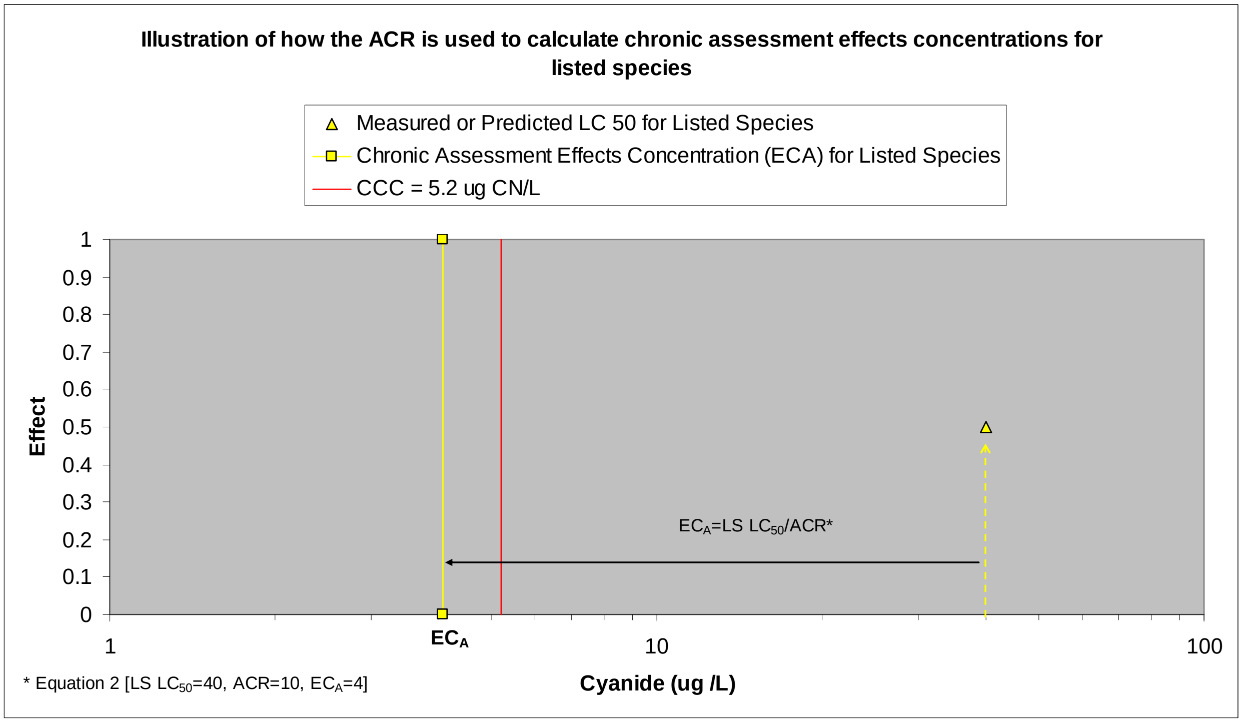


Figure 3. Illustration of how the Chronic Assessment Effects concentrations (EC_A) may be estimated using the surrogate species NOEC and the ratio of the surrogate species LC_{50} to the listed species LC_{50}.

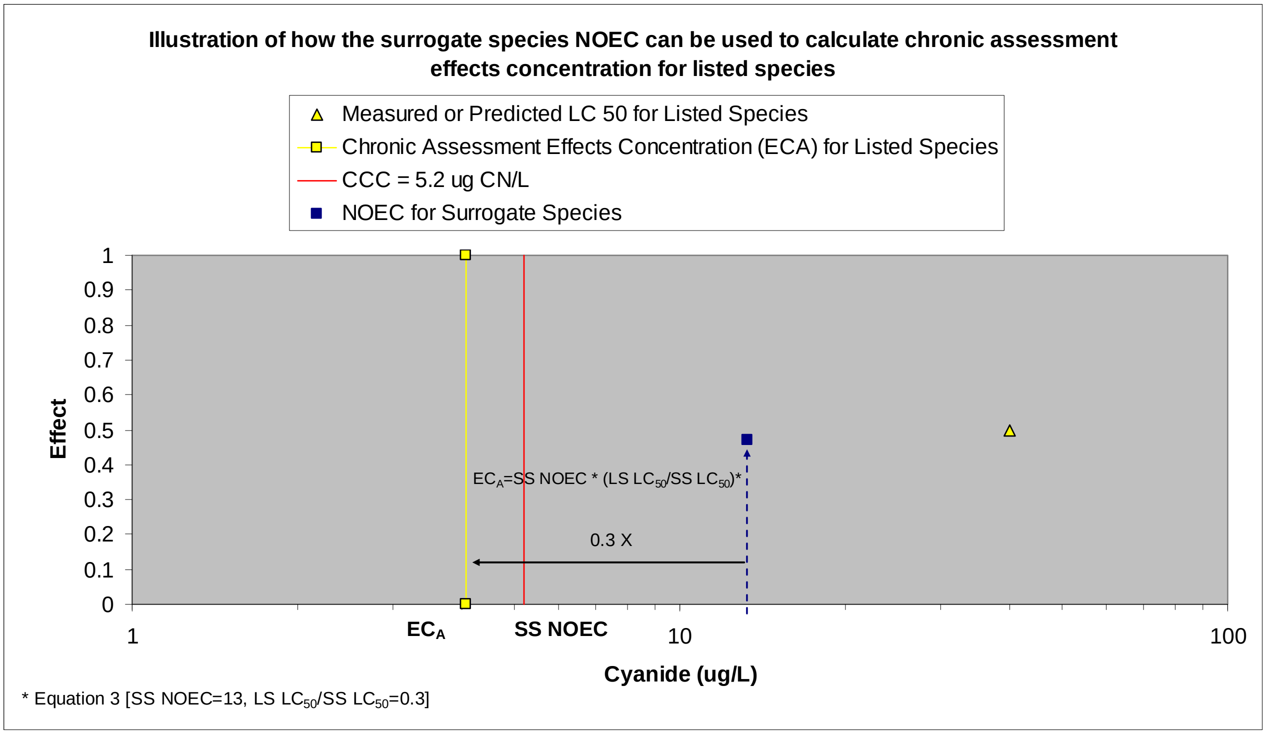
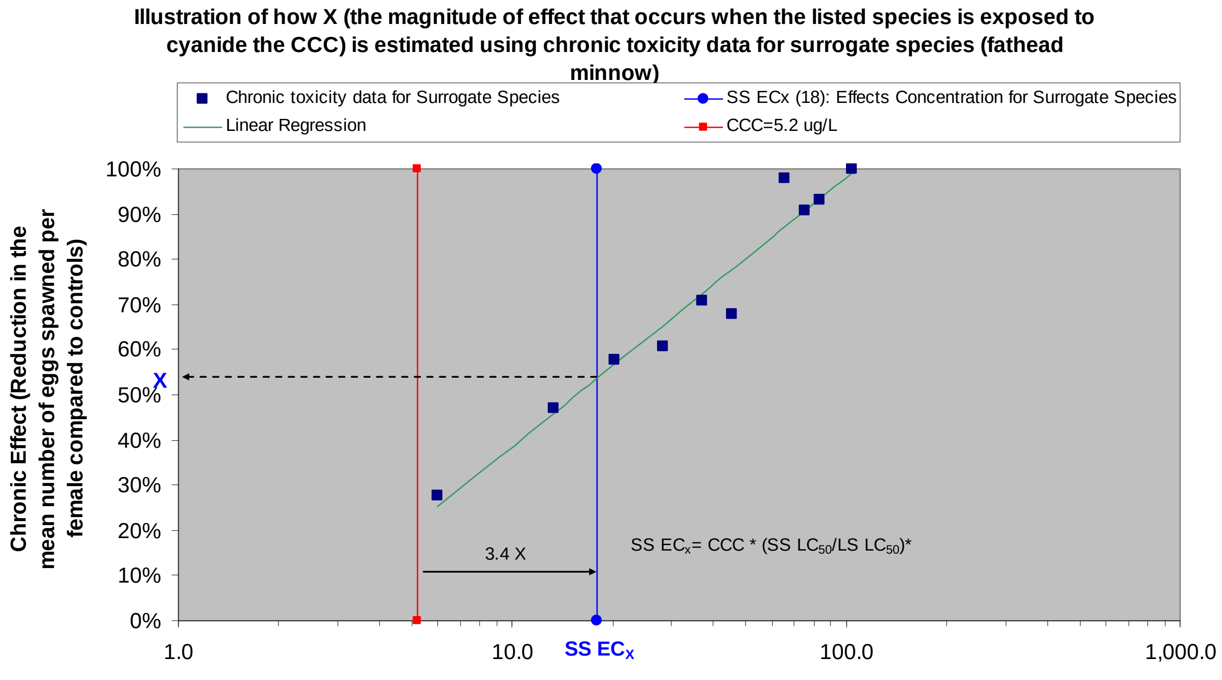


Figure 4. Illustration of how the magnitude of effect may be estimated using exposure – response data for surrogate species.



* Equation 5 [CCC=5.2, SS LC_{50}/LS LC_{50}=3.4, SS EC_x=18]

Recalculation of the Lethality Threshold Adjustment Factor (LTAF) for Fish

EPA's (2007) final Biological Evaluation (BE) identified 31 species of fish and 1 species of invertebrate for which the acute effects assessment concentrations (EC_As) were lower than the current acute (CMC) criterion for cyanide of 22.4 µg CN/L, as listed below (from EPA 2007:Table 4):

Species: common name	**Species: scientific name**	**BE Acute EC_a (ug CN/L)**
FISH:		
Amber Darter	*Percina antesella*	20.04 (ICE-Percidae)
Apache Trout	*Oncorhynchus apache*	9.08 (ICE-*O. apache*)
Bayou Darter	*Etheostoma rubrum*	18.93 (ICE-*Etheostoma*)
Bluemask Darter	*Etheostoma sp.*	18.93 (ICE-*Etheostoma*)
Boulder Darter	*Etheostoma wapiti*	18.93 (ICE-*Etheostoma*)
Bull Trout	*Salvelinus confluentus*	8.62 (ICE-*Salvelinus*)
Cherokee Darter	*Etheostoma scotti*	18.93 (ICE-*Etheostoma*)
Chinook Salmon	*Oncorhynchus tshawytscha*	16.26 (ICE-*O. tshawytscha*)
Chum Salmon	*Oncorhynchus keta*	21.41 (ICE-*Oncorhynchus*)
Coho Salmon	*Oncorhynchus kisutch*	15.51 (ICE-*O. kisutch*)
Conasauga logperch	*Percina jenkinsi*	20.04 (ICE-Percidae)
Duskytail Darter	*Etheostoma percnurum*	18.93 (ICE-*Etheostoma*)
Etowah Darter	*Etheostoma etowahae*	18.93 (ICE-*Etheostoma*)
Fountain Darter	*Etheostoma fonticola*	11.33 (ICE-*E. fonticola*)
Gila Trout	*Oncorhynchus gilae*	21.41 (ICE-*Oncorhynchus*)
Goldline Darter	*Percina aurolineata*	20.04 (ICE-Percidae)
Greenback Cutthroat Trout	*Oncorhynchus clarki stomias*	21.41 (ICE-*Oncorhynchus*)
Lahontan Cutthroat Trout	*Oncorhynchus clarki henshawi*	11.85 (ICE-*O. c. henshawi*)
Leopard Darter	*Percina pantherina*	20.04 (ICE-Percidae)
Little Kern Trout	*O. aguabonita whitei*	21.41 (ICE-*Oncorhynchus*)
Maryland Darter	*Etheostoma sellare*	18.93 (ICE-*Etheostoma*)
Niangua Darter	*Etheostoma nianguae*	18.93 (ICE-*Etheostoma*)
Okaloosa Darter	*Etheostoma okaloosae*	18.93 (ICE-*Etheostoma*)
Paiute Cutthroat Trout	*Oncorhynchus clarki seleniris*	21.41 (ICE-*Oncorhynchus*)
Relict Darter	*Etheostoma chienense*	18.93 (ICE-*Etheostoma*)
Roanoke Logperch	*Percina rex*	20.04 (ICE-Percidae)
Slackwater Darter	*Etheostoma boschungi*	18.93 (ICE-*Etheostoma*)
Snail Darter	Percina tanasi	20.04 (ICE-Percidae)
Sockeye Salmon	*Oncorhynchus nerka*	21.41 (ICE-*Oncorhynchus*)
Spotfin Chub	*Cyprinella monacha*	18.50 (ICE-*C. monacha*)
Watercress Snail Darter	*Etheostoma nuchale*	18.93 (ICE-*Etheostoma*)
INVERTEBRATES:		
Illinois Cave Amphipod	*Gammarus acherondytes*	15.33 (ICE-*Gammarus*)

Subsequent to the submission of EPA's (2007) final BE, the Vermilion Darter (*Etheostoma chermocki*) was added to the species list for this consultation and, along with most other *Etheostoma* darters, presumably would have been assigned an acute effects assessment concentration (EC_A) of 18.93 ug CN/L. That brings up to 32 the number of fish species initially warranting an acute effects analysis.

None of the acute EC_As that fell below the current acute (CMC) criterion for cyanide were derived from directly measured exposure-response curves for acute exposures to cyanide among any of the 32 species of fish and 1 species of invertebrate listed above. All of the EC_As in question were estimated from eleven ICE (Interspecies Correlation Estimates) models matching eleven taxonomic groupings (such as *Etheostoma* darters) of the species listed above. EPA (2007) derived the BE EC_As by calculating lower 90% confidence limit values for ICE-estimated acute LC_{50}s and then dividing those surrogate LC_{50} estimates by a lethality threshold adjustment factor (LTAF) of 2.27 to adjust the expected effects level downward from 50% lethality to a level estimated to fall somewhere between 0-10% (EPA 2006).

The LTAF of 2.27 is based on a compilation of data (n=219) for an assortment of chemicals, effluent waters of unknown chemistry, and test species that was published by EPA in the May 18, 1978 *Federal Register* (43 FR 21506). In Section 3.3.1.1 of EPA's (2006) *Draft Framework for Conducting Biological Evaluations of Aquatic Life Criteria, Methods Manual* it is recommended, if possible, that the generic LTAF of 2.27 be reviewed for appropriateness when applied to particular chemicals and species of receptor organisms. Such a review was not part of EPA's (2007) final BE. However, it was noted in Gensemer et al. (2007) that a LTAF substantively lower than 2.27 appeared to be warranted based on response data for acute exposures of Rainbow Trout to aqueous cyanide.

Review of 1978 LTAF data compilation for applicability to cyanide: An examination of the 219 LTAFs published in 1978 (43 FR 21506) revealed that none of those data came from studies of cyanide acute toxicity. It also revealed that there was no standardization of the "threshold" effect level associated with the compiled LTAF values. The adjustment factors were believed to vary from LC_{50}/LC_{01} to LC_{50}/LC_{10} ratios. Due to such variable "threshold" reference points, along with the other sources of variability inherent in a universally pooled sample of multiple chemicals and multiple test organisms, the reported estimates of LTAFs ranged from as low as 1.10 to as high as 50. Clearly, applying the geometric mean (2.27) of such a broad range of candidate LTAFs introduces a substantive source of uncertainty into estimates of EC_As.

Calculating EC_{10} standardized cyanide-specific LTAFs for fish: Subsequent to EPA's (1978) *Federal Register* publication of the generic LTAF data compilation, cyanide-specific data for several species of fish and life stages were published by Smith et al. (1978) and Broderius and Smith (1979). Furthermore, these authors published acute exposure-response regression equations which provide a basis for calculating standardized LTAF estimates.

If data were statistically powerful enough to support it, LTAFs ideally should be standardized to an LC_{50}/LC_{01} ratio. Existing data, however, are not statistically powerful enough to clearly separate an LC_{01} level of toxic response from the control response. As also noted for the chronic toxicity effects assessment in this biological opinion, the next best alternative is to standardize to an LC_{50}/LC_{10} ratio. As noted by Dwyer et al. (2005), a 10% level of control mortality is considered acceptable for toxicity test designs used to generate acute toxicity data such as presented in Smith et al. (1978) and Broderius and Smith (1979). Although, this biological opinion standardizes re-calculated LTAFs to the LC_{10} level of acute toxic response, it should be clearly understood that this is a statistical compromise. Whenever best available data can support a more statistically powerful estimate of toxic thresholds, such as the LC_{05} or LC_{01}, those alternatives would be preferred in the Section 7 context as using these values would reduce the chance of making a not likely to adversely affect determination, when an adverse effect exists.

There are 62 acute exposure-response regression equations from which LC_{50}/LC_{10}-standardized estimates of LTAFs can be calculated (Appendix G). Results of those calculations can be summarized as follows:

Life Stage	Species	Mean LC_{50}/LC_{10} LTAFs
Eggs / Sac Fry:	Fathead Minnow n=5	1.89
	Brook Trout n=4	2.09
	GM of spp. means	**1.99**
Fry:	Fathead Minnow n=5	1.55
	Bluegill n=4	2.09
	Brook Trout n=5	1.40
	GM of spp. means	**1.66**
Juvenile:	Fathead Minnow n=16	1.28
	Bluegill n=7	1.23
	Yellow Perch n=6	1.24
	Non-salmonid spp. GM	**1.25**
	Brook Trout n=9	1.15
	Rainbow Trout n=1	1.14
	Salmonid spp. GM	**1.14**
	Pooled Fish spp. GM	**1.21**

As reviewed by Eisler (2000), for fish the juvenile life stage is more sensitive to cyanide than the egg, sac fry or fry life stages. EPA's guidelines for deriving water quality criteria (Stephan et al. 1985) stipulate that they be derived from toxicity test data for the most sensitive life stage. Accordingly, the cyanide-specific, and LC_{50}/LC_{10}-standardized, LTAF results presented above for the juvenile life stage are the most applicable values for recalculating acute EC_As. Those values are substantively lower than the generic LTAF value of 2.27 from the 1978 *Federal Register (*43 FR 21506) data compilation. As a result, recalculated EC_As using this new LTAF suggest that more species could have been screened out as not likely to experience lethal effects when exposed to cyanide at the CMC.